Site-67 Substitutions of Cytochrome *c* by Semisynthesis: Implications for Structure and Function

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Cytochrome *c* is an electron transfer protein of the mitochondrial respiratory chain. It is a small metalloprotein of approximately 12,400 Daltons with a covalently attached Fe^{2+} Protoporphyrin IX prosthetic group [1]. Study of the *c* type cytochromes has been particularly intense and recent interest has centered on determining the role of specific amino acid residues in the protein. Recent reports have explored the nature of analogs prepared by substitution of one residue for another at a number of different sites [2].

Preparation of cytochrome *c* analogs with substitutions at residue 67, a tyrosine (Tyr) in the native protein, was undertaken in the hope of defining the role of this residue. Located in the hydrophobic interior of the protein near the heme, Tyr-67 is thought to be involved in important hydrogen bonding interactions with the sixth ligand to the heme iron and a buried water molecule [3]. Consequently, substitutions which would alter or eliminate the hydrogen bonding ability of Tyr-67 were examined.

Semisynthetic techniques, combining native and synthetic peptides, are particularly well suited to studies of this type and were used in this work. An important advantage of semisynthesis over other techniques such as site-directed mutagenesis, is that it allows incorporation of non-physiological amino acids. Preparation of the analogs was achieved by cleaving the protein with cyanogen bromide (CNBr), a reagent which cuts the protein after methionine residues. Although cytochrome *c* has two methionines, under limiting conditions, cleavage was restricted to methionine 65, allowing separation of a heme-containing peptide with residues 1-65 from the reaction mixture. Synthetic peptides having the desired substitutions at residue 67 were prepared by solid phase peptide synthesis and coupled to the native heme peptide to yield intact protein analogs. In this way, analogs with phenylalanine (Phe) and p-F-phenylalanine (p-F-Phe) at position 67 were prepared. Phe-67 lacks the ability to hydrogen bond (-H), while p-F-Phe-67 has altered hydrogen bonding ability (-F) compared to the native Tyr residue (-OH). The site-67 analogs were characterized by a variety of methods, including visible and circular dichroism spectroscopies, redox potential determinations, biological assays with cytochrome *c* oxidase, and ligand binding. Results of these characterizations, comparisons to the native protein and an appropriate control analog, and possible explanations of the observed differences on the basis of changes in hydrogen bonding will be discussed.

References

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